REMARKS

The Office Action and the cited and applied references have been carefully studied. No claim is allowed. Claims 1, 7-10, 12-16, 25, 28, 29, 31-42, 44, and 46-55 presently appear in this application and define patentable subject matter warranting their allowance. Reconsideration and allowance are hereby respectfully solicited.

The three double patenting rejections below will be discussed together.

1. Claims 29-20 and 33-34 are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claim 1 of U.S. Patent No. 5,824,543. The examiner holds that the presently claimed isolated DNA comprising a kidney-specific promoter (i.e., uromodulin) operatively linked to a heterologous DNA sequence encoding a heterologous polypeptide and directs expression of the polypeptide in the kidneys is not patentably distinct from "a recombinant nucleic acid construct comprising a mouse UP-II promoter operatively linked to a heterologous gene wherein the promoter directs expression of the heterologous gene product in the urothelium" as claimed in U.S. Patent No. 5,824,543.

2. Claims 29-34 are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claim 1 of U.S. Patent No. 6,001,646. The examiner states that the presently claimed isolated DNA comprising a kidney-specific promoter (mouse or goat uromodulin) operably linked to a heterologous DNA sequence encoding a heterologous polypeptide and directs expression of the polypeptide in the kidneys is not patentably distinct from "a vector comprising a UP-II promoter operatively linked to a heterologous

gene encoding a selected biologically active molecule wherein the promoter directs the expression of the heterologous gene product in the urothelium" as claimed in U.S. Patent No. 6,001,646.

3. Claims 29-34 and 38-46 are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-8 of U.S. Patent No. 6,339,183. The examiner asserts that, although the conflicting claims are not identical, they are not patentably distinct from each other because an isolated DNA molecule comprising a kidney specific promoter (mouse or goat uromodulin) operably linked to a heterologous DNA sequence encoding a heterologous polypeptide and directs expression of the polypeptide in the kidneys as presently claimed encompasses the subject matter of "a vector comprising a promoter construct linked to a heterologous DNA encoding a selected biologically active molecule wherein the promoter construct linked to a heterologous DNA encoding a selected biologically active molecule, wherein the promoter construct directs expression of the heterologous DNA to the urothelium so that the selected biologically active molecule expressed by the heterologous DNA is detected in the urine" as claimed in U.S. Patent No. 6,339,183.

The above three double patenting rejections are respectfully traversed.

It is well recognized in the art that uromodulin is distinctly different from uroplakin II (UPII) as a protein and in its encoding DNA, promoter, functional properties, and tissue specificity. Uromodulin is synthesized by the epithelial cells of the ascending limb of Henle's loop and the beginning portion of the distal convoluted tubule (and delivered exclusively to apical membrane and secreted into

the urine) but not by the epithelial cells of the urothelium which includes bladder epithelium. Zhu et al, Am. J. Physiol. Renal Physiol. 282:F608-F617 (2002), a copy of which is attached hereto, demonstrates on page F613, left column, that transgene expression of EGFP from a uromodulin (also known a Tamm-Horsfall protein or THP) promoter is consistent with localization at the thick ascending limb of Henle's loop (TALH) and early distal tubules. Expression, as measured by green fluorescence, was however not detected in any other tissue examined, including urinary bladder (urothelium), which is as expected if uromodulin and its promoter are kidney-specific and not urothelium-specific.

Conversely, uroplakins, such as UPII, are expressed in the urothelium but not in the tubules of the kidney. As disclosed on page 682, left column in the same Lin et al. (PNA, 1995) reference cited and applied by the examiner in the §102(b) rejection discussed below:

> Epithelia closely related to the epithelium of the bladder are known to cover other areas of the urinary tract, including the renal pelvis of the kidney, the ureter and the urethra. All those epithelia (known collectively as urothelium) share the common feature of elaborating AUM plaques as their terminal differentiation products.

The expression of reporter gene driven by the UPII promoter was found in all these urothelia but was not detected in any non-urothelial epithelia. A large amount of UPII PCR product from mRNAs was generated from the bladder but not from the kidney. While the urothelium extends to the renal pelvis of the kidney into which the urine flows from the tubules before reaching the urinary tract and bladder, uroplakins are not expressed in the rest of the kidneys and are certainly not kidney-

specific. Furthermore, in addition to their expression in very different organs, the promoters of uromodulin and UP-II and the protein products of the two genes share no sequence homology. Their "chemical compositions" are drastically different. Therefore, the expression of recombinant proteins in the kidneys and expression of recombinant proteins in the bladder are mutually exclusive and neither one encompasses nor makes each other obvious. Accordingly, there is no obviousness-type double patenting between the present claims and the claims of U.S. Patent Nos. 5,824,543; 6,001,646; or 6,339,183.

Reconsideration and withdrawal of the double patenting rejection are therefore respectfully requested.

Claims 25, 29-30, 33-34, 36-41 and 44 have been rejected under 35 U.S.C. §102(b) as being anticipated by Lin et al., PNAS 92:679-683 (1995). This rejection is respectfully traversed.

As discussed above in applicant's remarks directed to the double patenting rejections, Lin discloses that the expression of the β-galactosidase reporter gene driven by the UPII promoter was found in all urothelia but was not detected in any non-urothelial epithelia. Furthermore, UPII mRNA, as determined by PCR, was detected in the bladder but not in the kidneys. Accordingly, Lin cannot anticipate the presently claimed invention.

The examiner has also taken the position that it is known in the art that urothelium (uroepithelium) is involved in excretion of urine especially the uromodulin which is regulated by the uromodulin promoter, citing Su et al. (1997). However, a reading of Su et al. reveals nothing about the expression of uromodulin by the urothelium. Rather, Su et al. clearly disclose in their introduction that

uromodulin is "synthesized by epithelial cells of the ascending limb of the loops of Henele and the adjacent segment of the convoluted tubule".

Reconsideration and withdrawal of the rejection are therefore respectfully requested.

Claims 1, 8-16, 25, 28-29 and 38-47 have been rejected under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. The examiner states that the specification fails to disclose recombinant DNA molecules comprising all kidney specific promoters, all non-native apical surface membrane targeting sequences, all protease sensitive linkers, all protein having basolateral surface membrane targeting signals. In addition the examiner asserts that the specification fails to disclose a single non-human transgenic mammal that encodes a transgene comprising the above mentioned components so that the animal excretes any heterologous polypeptide of interest in the urine of the mammal. This rejection is respectfully traversed.

Applicant does not concede that there is a lack of written description for kidney-specific promoters. However, to advance applicant's business strategy, the claims are amended to delete the recitation of "kidney-specific promoter" and instead recite a "uromodulin" promoter, which is the preferred embodiment of a kidney-specific promoter. The sequences of the mouse and goat uromodulin promoters are disclosed in the specification. Furthermore, the bovine and rat uromodulin promoter regions have already been identified prior to the time the present invention was made. See the specification at

page 18, lines 15-19, where Yu et al. (1994) is cited for the sequences of the bovine and rat uromodulin promoter regions.

The specification at pages 26 to 28 discloses GPI signal sequences as apical surface membrane targeting sequences where uromodulin GPI signal sequences and GPI signal sequences from other proteins are disclosed and identified by SEQ ID NOs. Furthermore, the present specification at pages 31 to 32 teaches Asn-linked glycosylation and O-glycosylation as apical surface membrane targeting sequences. Accordingly, a representative number of apical surface membrane targeting sequences are disclosed in the specification, thereby satisfying the written description requirement.

Applicants respectfully point out that the presence of a non-native apical surface membrane targeting sequence or the inactivation of deletion of a basolateral surface membrane targeting sequence do not have to be universally used. These are signals that can be added, modified, inactivated or deleted, if need be, in the event that a particular desired heterologous polypeptide is not targeted to the apical surface membrane or leaks through the basolacteral surface membrane as discussed in the specification at pages 24-27.

Those of skill in the art are well aware of chemically or enzymatically cleavable (i.e., protease-sensitive) sequences and linkers containing such sequences that can be used to separate the different components of a fusion protein. Fusion proteins have been commonly used in the prior art for a long time and the cleavage sites, such as would be provided by a linker between fusion components, are well known to those of skill in the art.

Regarding the examiner's position that the specification fails to disclose a single non-human transgenic mammal that encodes a transgene comprising the above mentioned components so that the animal excretes any heterologous polypeptide of interest in the urine of the mammal, applicant discloses in the specification at page 11, lines 17-18 that farm animals such as pigs, sheep, goats, horses and cattle may be used as the transgenic kidney-based urinary bioreactor of the present invention. To satisfy the written description requirement, that is all that is needed. There is no requirement that a single non-human transgenic mammal comprising all the components and embodiments of the present invention be actually obtained. Prophetic examples are certainly acceptable.

Reconsideration and withdrawal of the rejection are therefore respectfully requested.

Claims 1, 7-10, 12-16, 25, 28-47 are rejected under 35 U.S.C. §112, first paragraph, because the examiner states that the specification, while being enabling for an isolated DNA molecule comprising a mammalian uromodulin promoter operably linked to a fusion polypeptide comprising heterologous DNA sequence encoding a heterologous polypeptide containing the uromodulin GPI sequences for apical surface membrane targeting sequence, does not reasonably provide enablement for any DNA molecule that contains any and all kidney specific promoters, non-native apical surface membrane targeting sequences, protease sensitive linkers, modified basolateral surface membrane targeting signals and any and all non-human transgenic animals encoding the DNA molecules (as claimed) wherein the heterologous

polypeptide of interest is produced in the urine. This rejection is respectfully traversed.

Applicant agrees that genetic background can sometimes influence a particular phenotype. However, the presently claimed invention has very little to do with phenotypic analyses of transgenic animals. Recombinant proteins are to be expressed under the control of the uromodulin promoter and secreted into the urine, where recombinant proteins have very little impact on animal phenotype. For example, the goal of expressing human growth hormone in the urine is not to study the effect of hGH on mouse growth. Whether a mouse expressing hGH becomes obese or not has no direct bearing on the fact that their kidneys secrete hGH into the urine which can be isolated for commercial use.

It cannot be ruled out that perhaps some recombinant proteins will be incorporated into the basolateral membranes (adjacent to blood vessels) of the kidney epithelial cells and then be leaked into the bloodstream causing side effects. However, this leakage problem can be minimized or eliminated by directing the recombinant proteins to the apical surface membrane (adjacent to urine in the tubules). Preferred embodiments of the present invention are directed to supplementing the recombinant proteins with apical surface membrane targeting signals such as GPI, glycosylation sites or by deleting basolateral targeting signals.

The position effect of transgenes has been reported. The general consensus is that the host genes adjacent to the transgene can positively or negatively affect transgene expression. For example, a strong enhancer would stimulate transgene expression and a strong

silencer would suppress the expression. However, this is not an insurmountable problem. For instance, adding "insulation sequences" or nuclear matrix attachment region at both ends of a transgene can sufficiently prevent position-dependent effects (Thorey et al., Mol. Cell Biol. 13(11):6742-6751, 1993; Willoughby et al., J. Biol. Chem. 275(2):759-768, 2000, a copy of which is attached hereto; Lee et al., Mol. Cells, 8(5):530-536, 1998). Some promoters also have natural insulation sequences, thus circumventing the need of adding extra ones (Zhu et al., Am. J. Physiol. 282:F608-F617, 2002, a copy of which is attached hereto; Lin et al., PNAS, 92:679-683, 1995, cited by the examiner). Since the position effect occurs mostly randomly (as transgene insertion is a random event), this position effect can also be minimized by the development of multiple transgenic lines, some of which do not at all exhibit insertion site-dependent effects (Zhang et al., Cancer Res. 59:3512-3517, 1999; Zhang et al., Oncogene, 20:1973-1980, 2001; Zhu et al., Transgenic Res. 12;155-162, 2003, copies of which are attached hereto).

How many intrinsic elements of a gene are required for tissue-specific and high-level expression depends on a given gene. Applicant's own evidence indicates that the 3.0 kb of 5'-upstream sequence are sufficient to direct kidney-specific and high-level expression (Zhu, et al., 2002; 2003). A recent report by Kim et al. Transgenic Res. 12:191-201 (2003), a copy of which is attached hereto, showed that only 600 base pairs of the bovine uromodulin gene are required for kidney-specific expression. This demonstrates that Zbiskowska et al.'s disclosure (cited by the examiner) of using a modified uromodulin promoter that contains exon 1 and a part of exon 2

to provide kidney specific expression in transgenic mice that leads to production of the recombinant protein in the urine was merely due to their arbitrary selection of a fragment containing a uromodulin promoter. As is clear from the Zhu et al. (2002, 2003) and Kim et al. (2003) references discussed above, only 5'sequences upstream of the uromodulin coding sequences are needed to direct kidney-specific expression.

Furthermore, Zbikowska et al. cited by the examiner discloses that a human uromodulin promoter functions in mouse to direct expression of biologically active human α-antitrypsin into the urine. Thus, the fact that a human uromodulin promoter is interchangeable with a mouse uromodulin promoter to direct expression to the urine as demonstrated by Zbikowska lends supports to the disclosure in the specification at page 17, last paragraph, that "the uromodulin promoter from one mammal species is believed to be functional in another species."

With respect to transgene incorporation efficiency, the success rates vary significantly from facility to facility. This is true even with transgenic mice. Methods are being continuously developed that dramatically improve the success rate. For example, Nottle and colleagues recently found that by increasing the amounts or concentration of transgene DNA the success rate of transgenic pigs increased from 4% to 26% (Nottle, et al., Transgenic Res. 10:523-531, 2003, a copy of which is attached). These rates approximate those with transgenic mice. With respect to kidney-based bioreactors, transgenic farm animals have additional advantages because of their large urine output. A transgenic mouse produces up to 1.5 ml of urine daily while

a transgenic cow produces up to 20 L daily (more than 10,000 fold as compared to mouse). At a minimum concentration of 500 ng/ml of recombinant protein, a cow can produce as much as 5 mg of a therapeutic protein daily or 1.9 g per year. A herd of 10 cattle could produce more than 18 g of protein yearly. This amount would be sufficient for a number of rare therapeutic proteins to satisfy the market needs of the entire country (Wall RJ et al., J. Dairy Sci., 80(9):2213-24, 1997; Zhu et al., Transgenic Res. 12:155-162, 2003, a copy of which is attached hereto). Furthermore, if it is routine to obtain transgenic laboratory animals like rabbits, mice and rats with a transgene efficiency of 3% as relied on by the examiner, then it would still be routine to obtain transgenic farm animals at a transgene efficiency of 1%. Even a single obtained transgenic farm animal is capable of producing a significant amount of a commercially desirable heterologous polypeptide.

Accordingly, the presently claimed invention is enabling to those of skill in the art. Reconsideration and withdrawal of the rejection are therefore respectfully requested.

Claims 8, 13, 15, 16, 34 and 37 have been rejected under 35 U.S.C. §112, second paragraph, as being indefinite. This rejection is respectfully traversed.

The examiner finds claim 8 unclear for what is "one or more non-native sites for glycosylation at predicated β-turns". The specification on page 31 discloses and teaches Asn-linked glycosylation and further teaches at the bottom of the page that, in order to maximize the likelihood of being glycosylated, one or more non-native glycosylation sites can be designed at predicted β turns in the

polypeptide, i.e., by the widely recognized Chou-Fasman algorithm or others commonly used in the art. Accordingly, this recitation is not indefinite.

Claim 13 is now dependent on claim 12 which provides antecedent basis for "said fusion polypeptide".

Claim 15 is amended to replace "disposed 3'" with "disposed downstream" as supported by the specification in the paragraph bridging pages 32 and 33 and as would be clearly understood by those of skill in the art.

The examiner states that claims 16 and 47 are indefinite as it is unclear how the "basolateral surface membrane targeting signals native to said heterologous polypeptide is inactivated or deleted". The specification in the paragraph bridging pages 33 and 34 discloses basolateral targeting signals and teaches that deletion or modification of the "tyrosine motif" or a double or di-leucine motif, i.e., by site-directed mutagenesis, leads to inactivation/blockage of basolateral targeting.

With regard to claim 34, it is clear from the specification at page 15, last paragraph, that a fragment of the uromodulin promoter intended to be encompassed in the claim is a fragment which retains the tissue specific promoter activity of uromodulin. As the nucleotide sequence of SEQ ID NO:1 in claim 34 contains more than just the uromodulin promoter, a fragment thereof would also retain the kidney-specific expression of uromodulin.

Reconsideration and withdrawal of the rejection are therefore respectfully requested.

In view of the above, the claims comply with 35 U.S.C. §112 and define patentable subject matter warranting their allowance.

Favorable consideration and early allowance are earnestly urged.

Respectfully submitted,

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